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Evaluation of an Enzyme-linked Immunosorbent Assay (ELISA) for the Direct Analysis of Molinate (Ordram®) in Rice Field Water

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A direct ELISA for the thiocarbamate herbicide molinate was used to study distribution and dissipation of the compound in a treated rice field. No sample preparation other than buffering and dilution was required for the analysis of field water samples. Analyses were performed in 96-well microplates and required less than 0·5 man-hour per sample (three dilutions per sample, four replicate wells per dilution). Spiked samples and selected field samples were split for analysis by ELISA and gas chromatography. Two control samples of 92 and 93 ppb (after dilution) had between run coefficients of variation of 13·8 and 13·9% for 37 ELISA runs. A nested ANOVA analysis revealed that the largest source of error for the ELISA was due to within replicate variability, partly attributable to interwell variability of the 96-well plates. Practical aspects of reducing assay error and handling ELISA data are discussed. Quality control data showed that reliability of the direct ELISA is comparable to the gas chromatography method for molinate. ELISA data from field samples showed concentration differences among sites in the same check which coincided with differences in water flow. The half-life of molinate in the field, as determined by ELISA, was comparable to the value determined by chromatography.

INTRODUCTION

Molinate (S-ethyl hexahydro-1H-azepine-1-carbothioate; trade name Ordram) is used to control water grass [*Echinochloa cruss-galli* (L.) Beauv.] in California rice fields. In 1985, 1 136 989 lbs of molinate active ingredient were applied in California (Califor-

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nia Department of Food and Agriculture, 1985). Fish kills attributed to molinate (Finlayson and Lew, 1983) have prompted concern over the levels of molinate in the drainage canals and the Sacramento River. The California Department of Food and Agriculture, California Department of Fish and Game, California Regional Water Quality Control Board (Central Valley Region), the City of Sacramento, and ICI, Inc. (formerly Stauffer Chemical Company and the manufacturer of molinate) have participated in an active monitoring programme for the past several years. Numerous samples can be generated by such a programme, and the analysis by classical analytical methods can be time-consuming and expensive. The gathering of data on molinate distribution in the environment has been partially limited by the cost of analysis and the lack of an appropriate screening method for molinate.

The search for rapid and cost effective pesticide analytical methods has been motivated by problems involved in analysing large numbers of samples and increased concern about the presence of pesticide residues in the environment and in foods. Immunochemical methods are more rapid and cost-effective than most classical methods and thus are well suited for use in screening programmes requiring the analysis of large numbers of samples. Numerous competitive immunoassays have been developed for the detection of pesticides (Newsome, 1986 and Van Emon *et al.*, 1988 for reviews; also Bushway *et al.*, 1988). The potential usefulness of immunochemical methods to environmental monitoring programs is becoming apparent to State and Federal agencies. One example of government commitment in this direction is the development of a direct competitive ELISA method for the detection of molinate in water (Gee *et al.*, 1988), under a research contract from the California Department of Food and Agriculture.

The present paper describes the application of this ELISA for the analysis of molinate in a situation representative of the expected use of the method for field monitoring. The objectives of this study were: (1) to test whether the ELISA method can be practically implemented for direct analysis (without a clean-up of extraction step) of water samples collected from a molinate-treated rice field; (2) to compare results obtained from both spiked and field samples by ELISA to those obtained by the accepted GLC method for precision and accuracy; and (3) to determine the spatial and temporal distribution of molinate in a treated rice field.

MATERIALS AND METHODS

Rice Field Treatment and Sampling. A laser leveled, commercial rice field in Colusa county, 6·4 km east of Maxwell, CA, served as a study site. The field consisted of 7 contiguous checks of 4·3 ha each. A field map with treatment and water flow history and sampling sites is given in Figure 1. On 10 May 1987, the field was treated with Ordram 10G (mixed before application with methyl parathion) by fixed wing aircraft at a rate of 4·5 kg molinate active ingredient per hectare. During the sampling period from 9 May through 22 May 1987, the pH and temperature of the water were recorded at the sampling sites. The pH ranged from 6·8 to 8·1 and the water temperature averaged 26°C (range 19 to 33°C). To maintain the water depth in the field the inlet was opened during the sixth day after application.

Sampling sites were located at each corner of the upper and lower checks. Water samples were collected one day before application and 1, 3, 6, 9 and 12 days after application. Quadruplicate samples were collected at each site from below the water surface and stored without transfer or filtering in 250 mL Kimax glass sampling bottles (Fisher Scientific). All bottles were frozen initially on dry ice, then stored at −20°C until analyzed.

Non-molinate treated rice field water was spiked with molinate at 0, 100, 500, 2000 and 10 000 ng/mL (seven replicates per level) and samples were split for blind analysis

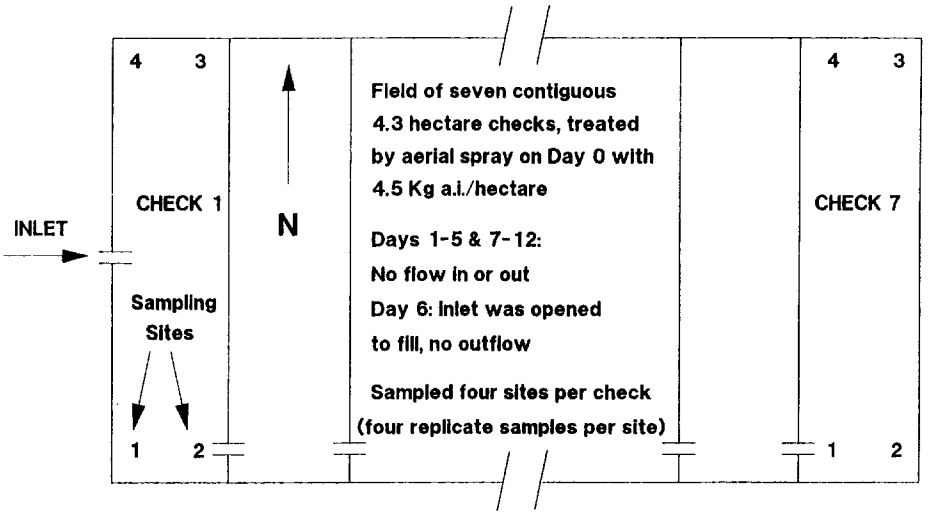

FIG. 1. Map of molinate treated field with water flow, treatment history, and sampling sites.

by both ELISA and GLC. Thirteen randomly selected field samples were also split and analyzed by both ELISA and GLC.

Sample Preparation and GLC Analysis. Samples were prepared for analysis according to the scheme shown in Figure 2. GLC analyses were performed using a Hewlett-Packard Model 5890A capillary gas chromatograph equipped with a nitrogen–phosphorus detector, a Hewlett-Packard Model 7672A Automatic Sampler and a Hewlett-Packard Model 3357 Laboratory Automation System. The column was a fused silica 15 m × 0·32 mm i.d. capillary column coated with 0·25 μm of crosslinked and bonded trifluoropropylsilicone. Oven temperature was held at 100°C for 0·5 min, then programmed for 100° to 220°C at 20°C/min. Injector temperature was 230°C and detector temperature was 300°C. Helium carrier gas was used at a flow rate of 2·4 mL/min. Sample volume injected was 1·0 μL using a splitless injection mode. Under these conditions, the retention time for molinate was approximately 3·4 min.

The chromatographic system was calibrated using solutions of known analyte content, 20 to 1000 ng/mL, prepared in toluene from analytical standards. External standard calibration was performed using the on-line data acquisition system and 1·0 μL injections of the 100 ng/mL standard. Calibration solutions between 1000 ng/mL and 20 ng/mL were then analyzed to establish linearity. If the analyte concentration was above the range of linearity, the sample was diluted to bring the concentration within the calibrated range of the instrument and the diluted extract was reanalyzed. The limit of detection under these analytical conditions was 1·0 ppb for molinate.

Quality assurance samples for the GLC method were analyzed by the same procedure as described above. The accuracy of the analytical method was established by analysis of control and fortified samples. Control samples, prepared from deionized water fortified with known amounts of the analyte, were extracted and the extracts analyzed to evaluate the efficiency of the extraction procedure and the accuracy of the instrumental analysis. Samples fortified with 2·0–2·5 ppb molinate were extracted and the extracts analyzed to evaluate recovery from the sample matrix. Recoveries range from 86 to 109%, with a mean recovery of 96%.

Laboratory blanks were analyzed in order to assure that the GLC method was free of contamination or interferences. Laboratory blanks of deionized water were

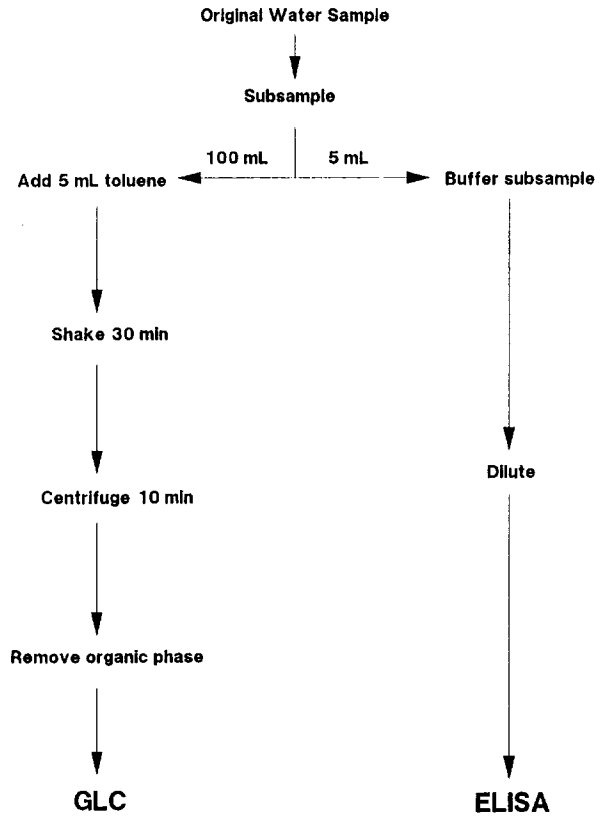

FIG. 2. Sample preparation for GLC and ELISA analysis of molinate in rice field water samples.

extracted and the extracts analyzed concurrently with samples. No molinate was detected in any of the laboratory blanks at a limit of detection of 1 ppb for molinate, indicating that contaminants or interferences were not important sources of error in the analysis.

Sample Preparation and ELISA Analysis. Samples were prepared for analysis according to the scheme shown in Figure 2. A generalized schematic of the sample handling and data flow is given in Figure 3. ELISA analysis was performed as described previously (Gee *et al.*, 1988) with the following exceptions. Multichannel pipetting for plate coating, sample transfer, and reagent addition was done with a 12 channel 50 to 200 μl pipettor (Flow Laboratories) and 96-well plates were washed with a 12 channel manual washer (Nunc). All multichannel pipetting operations were performed in the 'to deliver' mode, if possible, rather than in the 'to contain' mode. This allowed easy visual monitoring of interchannel volume differences during the operation and was a valuable quality assurance procedure. New 96-well microplates (Nunc #442404) were coated with 100 μL/well of a 2·5 μg/mL solution of coating antigen.

Standards or samples were prepared for analysis by incubating molinate (0–500 ng/mL) or sample dilutions with antiserum at a final dilution of 1/4000. Samples thus prepared for competitive inhibition were incubated overnight at 22°C in the wells of uncoated 96-well microplates (Dynatech) covered with adhesive plate sealers. After this competition step, four 50 μL aliquots were transferred from each well of the competition plate to each well of the antigen-coated plate (i.e. four replicate ELISA wells per inhibition well). The alkaline phosphatase substrate volume was

100 μL/well and plates were read in a Vmax microplate reader (Molecular Devices) using dual wavelength endpoint mode (405 nm–650 nm) with the reader's automatic mixing function turned on. The plate reader was interfaced to an IBM XT286 computer and data analysis was performed using the commercial software package Softmax (v. 1·01; Molecular Devices).

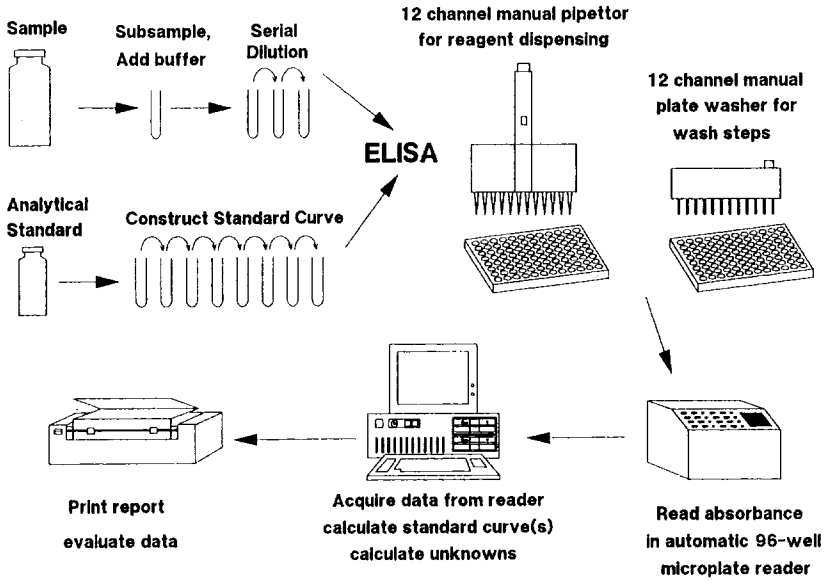

FIG. 3. Sample analysis and data flow schematic diagram for ELISA analysis of molinate in rice field water samples.

Standard curves of 500 to 7 ppb (twofold serial dilutions) plus zero ppb were run on each plate. Three control samples were also analyzed for quality assurance, including two field samples selected after preliminary ELISA analysis showed their concentrations to be appropriate for use as positive controls. These were thawed, aliquotted, and refrozen; on each day, an aliquot of each was thawed, diluted to approximately 100 ppb, and its concentration was determined by comparison to the standard curve for that plate. A negative control of buffer plus anti-molinate antibody, but with no molinate, was also run. This control was the same as the zero molinate standard, but was prepared independently and compared back to the standard curve as an unknown.

Field samples were buffered and diluted (three twofold dilutions per sample) for direct analysis in the ELISA. The decision making process used is summarized in Figure 4. If possible, an estimate was made of the dilutions needed for the sample value to fall within the standard curve. If no such estimate could be made, the sample was analyzed undiluted. Information on the dilution required for the first samples analyzed was used in choosing dilutions for other replicate samples from the same site, thereby minimizing the number of repeat assays.

The value reported for a sample was calculated from the dilution whose concentration fell closest to the C value (IC50; ppb giving 50% inhibition) of the standard curve, preferably greater than C, since this is the area of the standard curve with the least relative error (Rodbard, 1981; also see Results and Discussion, Figures 5 and 6). In some cases, well replicate CV for a sample dilution was also considered in the choice of which dilution to report. If two of the three sample dilutions chosen fell outside the 7 to 500 ppb range of the standard curve, the sample was assayed again with different

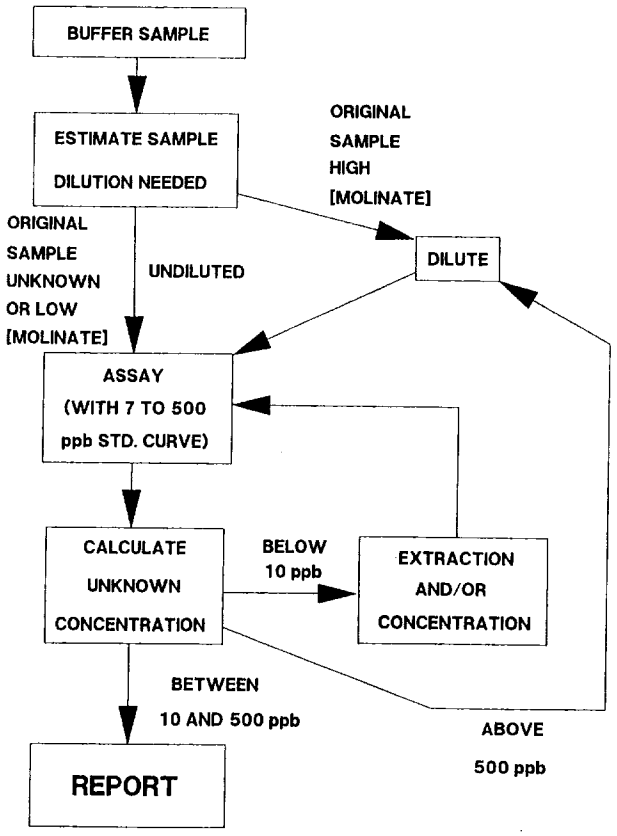

FIG. 4. Schematic diagram of decision-making process for ELISA analysis of molinate in rice field water samples. The extraction step for low concentration samples was not used in this study and is included to illustrate the interface with other methods (Li *et al.*, 1988).

dilutions. Diluted samples whose least diluted aliquot gave values at the low end of the standard curve (high relative error region of Figure 6) were routinely assayed again in less dilute form to obtain a more reliable measurement. Non-zero molinate concentrations below 20 ppb were not expected in this study, but for samples in this range, it is possible to integrate extraction and/or concentration schemes for use in ELISA (as indicated in Figure 4; Li *et al.*, 1988).

Statistical Analysis. Standard curve data were fitted to the four parameter logistic equation of Rodbard (1981) by the Molecular Devices software. The general form of the four parameter equation is

$$Y = \frac{(A-D)}{(1+(X/C)^B)} + D$$

where:

A = Response (absorbance) at zero dose
B = Curvature (i.e. approximate slope)
C = Concentration (ppb) giving 50% inhibition
D = Response (absorbance) at infinite dose

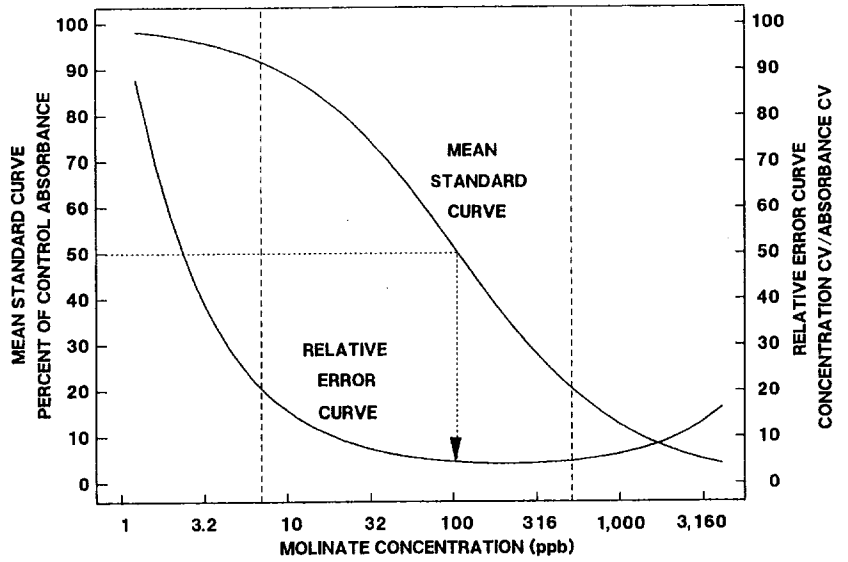

FIG. 5. Mean standard curve for 56 ELISA plates and the derived relative error function. Vertical dotted lines indicate the concentration limits for actual standard curves. Arrow indicates the mean IC_{50} of 106 ppb. The relative error curve represents the percent change in concentration estimate for each percent change in absorbance, plotted against concentration. Thus, the portion of the curve with the lowest relative error is from 100 to 500 ppb.

This equation was developed specifically for use in the analysis of sigmoid response curves, especially those seen in ligand binding assays such as immunoassays. Outlier removal was performed according to Youden and Steiner (1975). Regression analyses for comparing GLC and ELISA data were run using the SAS General Linear Models Program (SAS, 1985), with a confidence level of $p<0{\cdot}05$. Nested ANOVA procedures to isolate the sources of error were run using the SAS Nested ANOVA Program (SAS, 1985).

RESULTS AND DISCUSSION

Standard Curves. Figure 5 shows the mean standard curve for 56 assays, plotted as percentage of control absorbance. The mean standard curve was obtained by plotting the four parameter function given above using the $n=56$ means for each of the four parameters. Parameter means and standard deviations for 56 assays were (units as noted above in Materials and Methods): $A=0{\cdot}501\pm0{\cdot}070$, $B=0{\cdot}872\pm0{\cdot}135$, $C=105{\cdot}8\pm32{\cdot}1$, and $D=0{\cdot}151\pm0{\cdot}056$. The fit of the individual standard curves was excellent; the mean r was $0{\cdot}992\pm0{\cdot}007$. Figure 5 also gives the relative error curve derived from the mean standard curve, showing the relationship between assay precision and concentration. This curve was obtained by calculating the percentage change in the concentration estimate for each percentage change in the absorbance and plotting against concentration. Thus for a sample at 100 ppb, a decrease of 1% in absorbance would indicate an increase of 4% in the concentration estimate, while at 10 ppb a decrease of 1% in absorbance would indicate an increase of 15% in the concentration estimate. The relative error curve is a function both of the local slope of the standard curve and the analyte concentration and is therefore asymmetric.

Figure 6 shows the relative error curve of Figure 5 replotted against percentage of the control absorbance. This curve shows that the expected percentage error of the

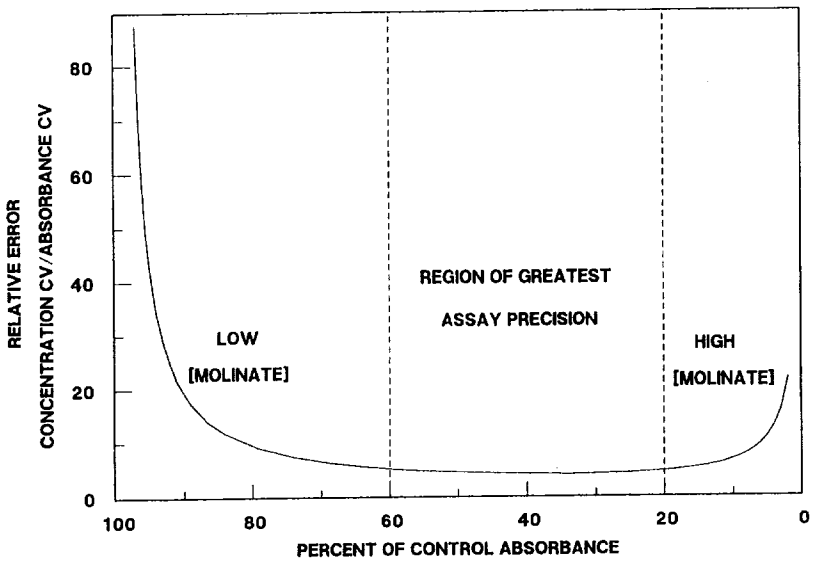

FIG. 6. Replotted relative error curve from Figure 5. This curve represents the percent change in concentration estimate for each percent change in absorbance, plotted against percent of control absorbance (linear X axis). Thus, the portion of the assay with the lowest relative error is from 20 to 60 percent of control absorbance.

concentration estimate is smallest in the region of 20–60% of the control absorbance. Therefore, values falling within this percentage of control range were considered more reliable estimates of sample concentration. The presentation of the relative error function given in Figure 6 is particularly well suited to serve as a visual guide to standard curve interpretation for inexperienced analysts.

An important lesson shown by Figure 5 is that the asymmetric nature of the error curve allows reasonable confidence measurements at somewhat higher concentrations than were made in this study. Thus the practical upper limit of the assay could have been extended from 500 ppb to as high as 3000 ppb simply by constructing the standard curve differently, by choosing a larger serial dilution factor and beginning the dilutions at a higher point. When preparing a protocol such as used for this study, it is best to define the lower tail of the standard curve as much as possible so that the usable range can be maximized.

Controls. Figures 7 and 8 are Shewhart charts of negative and positive control samples. The limit of reliable measurement was estimated at 21 ppb, approximately twice the $\bar{x}+2SD$ line of Figure 7, with $\alpha=\beta=0{\cdot}01$ (Wernimont & Spendley, 1985). It must be noted that this value is not directly comparable to commonly reported limits of detection because it considers the variability of both samples and blanks in the context of the entire analytical process, not just the instrument reading of a prepared sample. The limit of reliable measurement thus estimated is the lowest molinate concentration which can be distinguished from zero with respective risks $100\alpha\%$ and $100\beta\%$ of reporting false positives and false negatives. The limit of reliable measurement is probably more meaningful than a conventional limit of detection because it provides a conservative and statistically well supported estimate of the operating characteristics of the assay obtained under realistic conditions.

Close examination of Figures 7 and 8 reveals a slight negative trend in each. The second positive control (data not shown) also showed the same slight negative trend. In the case of Figure 7 this trend may reflect the experience of the analyst and

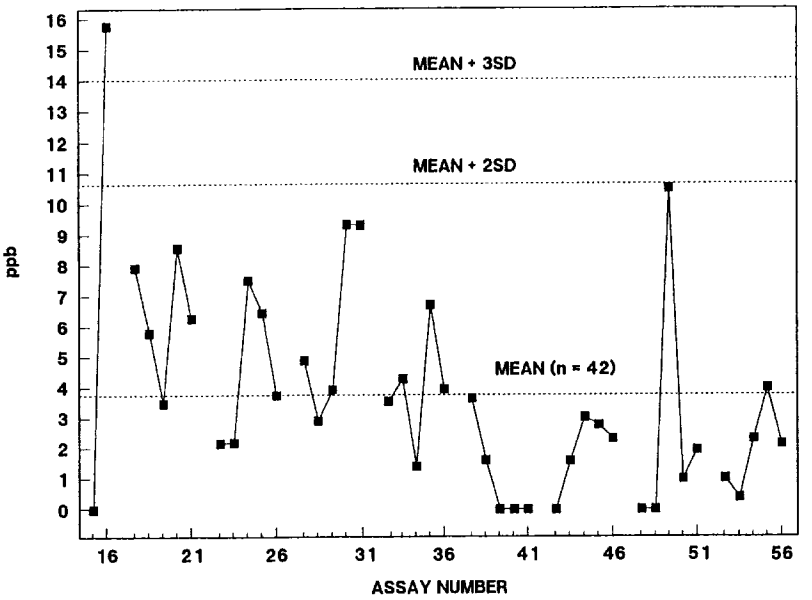

FIG. 7. Control chart for negative control sample (n=42). The limit of reliable measurement (99% confidence) is approximately twice the 10·6 ppb line ($\bar{x}$+2SD). Each point represents the mean of four replicate wells on one assay plate (one standard curve); connected points indicate assays run on the same day. Assays 1 to 14 are not plotted since they did not include this control.

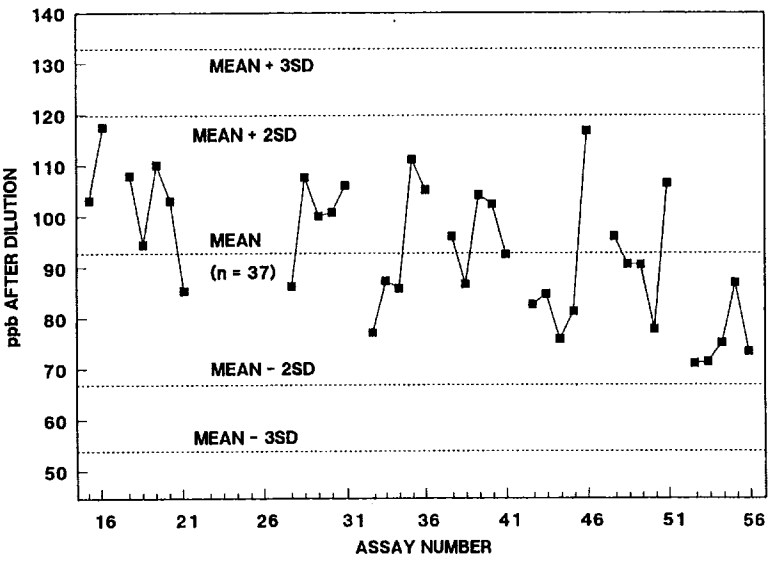

FIG. 8. Control chart for positive control sample (n=37). Each point represents the mean of four replicate wells on one assay plate (one standard curve); connected points indicate assays run on the same day. Assays 1 to 14 are not plotted since they did not include this control. Assays 22 to 26 were deleted because of procedural error during analysis.

TABLE 1. Results of nested ANOVA performed on two positive control samples; control number 1 corresponds to Figure 8.

Control number	Variance source	d.f.	Mean squares	Percent contribution
1	Total	146	577·30	100
	Day	7	1043·5	4·6
	Plate	29	559·37	0·3
	Replicate	110	552·36	95·1
2	Total	146	664·89	100
	Day	7	1571·4	8·3
	Plate	29	497·88	0·0
	Replicate	110	651·23	91·7

reduction of the assay noise level. In the case of the positive controls, the decrease in concentration may be due to volatilization of the molinate from the frozen control aliquots; samples were collected in May of 1987 and analyzed over a period of three months from December 1987 to March 1988. Despite these observations, neither positive control violated the quality control rules proposed by Westgard *et al.* (1981).

Procedural Sources of Error. Table 1 gives the results of a nested ANOVA for the two positive controls. By far the largest relative error contribution came from the ELISA replicate well level for both controls. Since the 4 replicates at this level were actually replicate ELISA determinations on one competitive inhibition sub-sample, it is likely that the observed error was introduced by one or more of the following factors: (1) interwell variability of antigen binding (plate coating) due to inherent variability of the plate surface properties; (2) pipetting error in the coating of the plate; (3) variability of subsequent reagent pipetting and washing; (4) variability of the 50 μL aliquot removed from the inhibition well, due to inadequate mixing or pipetting error.

Sources of error associated with the samples were not addressed in this study, but other errors inherent in the ELISA procedure were examined in an attempt to understand the relative contributions of each source, especially numbers (1) and (4) above. Interwell variability within plates (i.e. inherent plate noise plus pipetting, washing, and reader error) was estimated by simultaneous assay of 8 plates from one lot, treating all wells of all plates uniformly under the same conditions as the zero molinate control described in the Materials and Methods section. The mean of the 8 platewise CV values was $3{\cdot}8 \pm 0{\cdot}6\%$. The reader error component of this value (i.e. instrument imprecision plus inaccuracy) was 0·3% (reverse repeat well CV, Harrison and Hammock, 1988). Between-channel pipetting errors and single channel precision of the 12 channel pipettor used were less than 1%, measured gravimetrically using distilled water and a 0·1 mg balance. Manual plate washer performance was not evaluated statistically, but the volumes of wash buffer dispensed into separate tubes by each channel were not visibly different. In a comparison between the manual 12 channel Nunc washer used in this study and a Bio-Tek 403 96 channel automatic plate washer, the mean of the platewise CV values ($n=2$ for each washer) was 1·3% lower for the automatic washer. These data indicate significant contributions from all of source listed above, including the intrinsic interwell variability of the plates themselves. However, based on the experiments described above, we cannot determine the proportional contributions of the above sources. These data indicate that there is potential for improvement in the area of understanding of procedural sources of immunoassay error. A systematic study of all these variables together, in the context of an assay validation study, would be a valuable contribution to the immunoassay field.

Two other procedural variables, plate shaking and reading mode, were tested for their effect on assay precision. Use of the plate reader's built-in mixer to shake the plate immediately before reading reduced well replicate CV values as much as twofold. Dual wavelength reading (405 nm–650 nm) contributes a small but consistent reduction in replicate CV values (0·2%; $n=4$ plates). At the low colour development rates used (less than 20 mOD/min) kinetic reading gave 2 to 3 fold greater replicate CV values than endpoint reading ($n=4$ plates).

All the above error values are expressed in terms of percentage of Y (absorbance). The low slope (mean $B=0{\cdot}872$) of the standard curve magnifies small errors in Y into large errors in X. For the mean standard curve of Figure 5, each 1% change in the absorbance gives a 4·5% change in the concentration estimate at the 90 to 100 ppb concentration of the positive controls (the value of the Figure 5 relative error curve) and 4·0% at 225 ppb (65% inhibition), the most precise part of the mean standard curve. Thus, the interwell variability within plates of 3·8% would produce 17% variability in the concentration estimate in the most precise region of the mean standard curve.

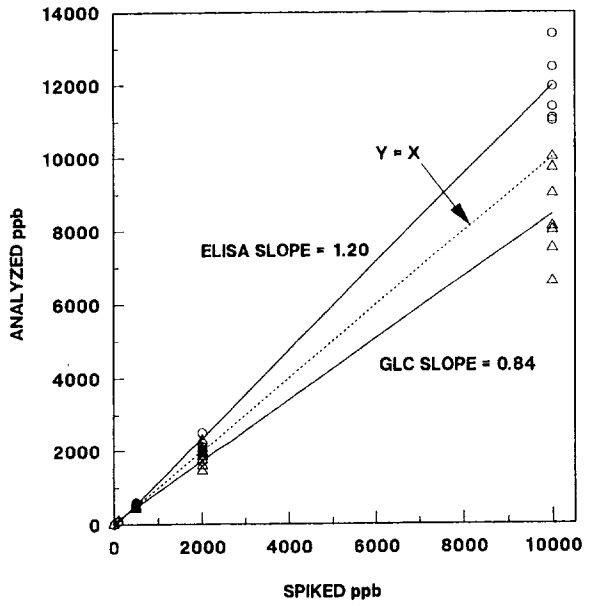

FIG. 9. Correlation between GLC and ELISA results for spiked samples at five concentrations, 6 or 7 replicate samples per concentration. Regression of results for each method was against the input spike values.

Correlation Between ELISA and GLC Methods. Figure 9 shows the correlation of GLC and ELISA values to spiked sample input values. The correlation between values determined for field samples by GLC and ELISA for 13 randomly chosen field samples is shown in Figure 10. All GLC samples were analyzed without replication and all ELISA samples were analyzed by the standard protocol described in the Materials and Methods section. The slope values from both figures indicate a slight high bias for the ELISA method relative to the GLC method. This bias was likely due in part to a matrix effect. Standard curves were constructed using buffered saline and samples were buffered using 1/10 part of 10X buffer. Additional salts present in the original

samples may have slightly decreased antibody binding, giving a falsely high concentration estimate (Li *et al.*, 1988).

Statistical analysis of the slopes from the spiked sample results of Figure 9 indicated that both ELISA and GLC methods were biased relative to the spike concentration. However, since concentrations of 10 000 ppb would not be expected in environmental samples, statistical evaluation was also conducted for the range of 0 to 2000 ppb. For this decreased concentration range, the ELISA line slope was 1·14 and the GLC line slope was 0·93. In this range, the ELISA line slope was significantly different than 1·00, but the slope for the GLC method was not.

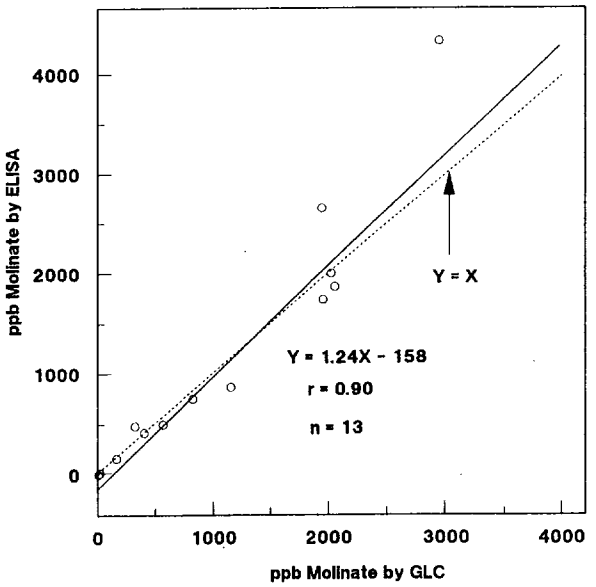

FIG. 10. Correlation between GLC and ELISA results for 13 randomly selected field samples.

Field Study Results. Table 2 shows the data obtained by ELISA on the various field samples. No molinate was detected in any of the pretreatment field samples. Relative values of sites within checks were consistent from day to day. Dilution in check 1 due to day 6 inflow was seen in the day 6 data, especially for sites 1 and 2, and in the day 9 and 12 data for all sites. On days 1 and 6 the values for opposite ends of check 1 were noticeably different, though in both cases the values for adjacent sites agreed. These within check differences can be reasonably explained, on day 1 by application differences, and on day 6 by preferential dilution at the end of the check having a connecting gate to the next check. In both of these cases the within check differences had decreased dramatically by the next sampling day, probably due to mixing within the check. The dissipation half life of 3 to 6 days was estimated graphically from day 1; 3, and 6 values and agreed with previous estimates (Ross and Sava, 1986).

Conclusions. This study clearly shows that ELISA can be used as a quantitative analytical technique to obtain precise and accurate data about molinate concentrations in field samples. The results of the analyses for molinate show no interference from methyl parathion, in agreement with the observed antibody crossreactivity (Gee *et al.*, 1988). The greatest advantages of this method are that the sample preparation time and effort are greatly reduced and that the analysis of samples is parallel rather than

TABLE 2. Molinate concentrations found by ELISA in field samples.

Day	Check #	Site #1	Site #2	Site #3	Site #4
Day 1	1	1510 (311)[a]	1472 (182)[c]	3815 (506)	3296 (136)[c]
	7	1735 (108)	2275 (690)	2319 (203)	2597 (992)
Day 3	1	1752 (283)	1461 (196)[b]	1575 (293)	2083 (512)
	7	1415 (101)	1854 (341)	1881 (180)	1279 (172)
Day 6	1	142 (10)[c]	125 (40)[c]	993 (246)	848 (111)
	7	961 (91)	1204 (330)	1004 (79)	1312 (380)
Day 9	1	239 (18)	156 (23)	368 (59)	448 (41)
	7	641 (46)	773 (99)[b]	679 (94)	682 (37)
Day 12	1	142 (29)[b]	99 (40)[b]	140 (25)	343 (81)
	7	370 (73)[b]	704 (84)[c]	620 (93)	539 (55)

[a] Values are the means of four replicate samples per site, unless noted otherwise; standard deviations are given in parentheses.
[b] Mean and standard deviation of three replicate samples.
[c] Mean and standard deviation of two replicate samples.

serial. These attributes have several important implications. It is easy to increase the sample load without drastically increasing the cost of analysis. ELISA is easily adapted to batchwise analysis; 20 samples per batch were run in this study with complete batch results in 24 hours using no automated equipment except for a microplate reader. The human attention required during this 24 hour period was less than 8 man-hours and the actual work was even less because a significant part of this was waiting during sample thawing and incubation periods. Batches of 80 samples per day could easily have been analysed under the study conditions if the samples were initially unfrozen, as would likely be the case in an active monitoring program.

ELISA can be adapted to nearly complete automation which can further decrease the cost while increasing sample loads. In plant pathology, where ELISA is used regularly for monitoring plant bacterial and viral diseases, one facility with advanced automation prepares and analyses 10–12 000 plant and seed samples/day (van Vuurde *et al.*, 1988). The automated operations required for a large-scale pesticide monitoring programme based on ELISA are not fundamentally different from those already proven in that plant and seed monitoring programme.

Another important attribute of ELISA is that although the sample preparation and GLC analysis are relatively easy in this matrix, the ELISA is still less expensive. For example, the cost per sample for the GLC method in this study was about $50 in-house and $130–200 when run by an outside contractor (assuming no replication and 10 samples/day). The ELISA, with three dilutions per sample and four replicate wells per dilution, costs $5–$6/sample (assuming 40 samples/day). This figure includes all costs except laboratory overhead and the cost of making the anti-molinate antibody. Antiserum cost is difficult to estimate in this case because it is the product of a research laboratory, but an overall cost of $5 to $15 sample would be consistent with the immunoassay kit products presently available commercially.

ELISA is easy to use, cost-effective, reliable, rapid, and readily automated. From a regulatory point of view, this is extremely valuable since it can be used to provide data that normally cannot be obtained in a timely, cost effective manner.

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REGISTRY NUMBERS

molinate	2212-67-1
methyl parathion	298-00-0

ABBREVIATIONS USED

ANOVA	analysis of variance
CV	coefficient of variation
ELISA	enzyme-linked immunosorbent assay
GLC	gas-liquid chromatography
IC50	concentration giving 50% inhibition
ppb	parts per billion (ng/mL)
SD	standard deviation
SAS	Statistical Analysis System

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